UNIVERSITÉ DE LAUSANNE — FACULTÉ DE MÉDECINE

Cell Junctions in Bowman's Capsule in Developing Rat and Human Kidney

THÈSE

préparée au Department of Anatomy,
University of British Columbia, Vancouver, Canada,
sous la direction du Professeur William A. Webber

et

présentée à la Faculté de médecine de l'Université de Lausanne
pour l'obtention du grade de docteur en médecine

par

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Médecin diplômé de la Confédération suisse
Originaire d'Oberwil im Simmental (Berne)

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IMPRIMATUR

Le Conseil de la Faculté de médecine de l'Université de Lausanne, après avoir pris connaissance des rapports favorables de MM. les professeurs G. Peters et W.-A. Webber, M. D., et sans se prononcer sur les opinions du candidat, autorise l'impression de la thèse intitulée : *Cell Junctions in Bowman's Capsule in Developing Rat and Human Kidney*, présentée par Mme Monique A. Ryser, médecin diplômé de la Confédération suisse pour l'obtention du grade de docteur en médecine.

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Cell Junctions in Bowman's Capsule in Developing Rat and Human Kidney *

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Summary. Electron-microscopic examination was made of the development of the cellular junctions in the parietal layer of Bowman's capsule in the kidney of the rat and of the human. In both species the developing junction differs from that seen in the mature individual in that it is relatively straight. The types of junctional specialization seen during development are similar to those present at maturity. Tight or gap junctions were not positively identified. Intermediate junctions with a relatively narrow intercellular space are common towards the apical end of the intercellular junction. Single or multiple desmosomes are frequently present deep to the intermediate junctions. The more basal regions of the intercellular junctions are unspecialized and show a space of 300 Å or more with occasional rather wide gaps. The junctional specializations are present even before adjacent cells have completed division and there is thus no morphological evidence for any changes of permeability of this layer during development. In addition the rat kidney appears to be a good model of the human with respect to the structure and development of the parietal layer of Bowman's capsule.

Key words: Kidney structure — Kidney development — Cell junctions — Bowman's capsule.

Introduction

The ability of adjacent cells to interact with each other has been investigated extensively (Loewenstein, 1972). The growth, the differentiation and the formation of mechanically and physiologically coherent tissues can be said to depend in great part on the ability of adjoining cells to establish a specialized form of communication, the cellular junction. A number of types of junctional specialization have been described in the literature including junctional complexes (Farquhar and Palade, 1963; Fawcett, 1963; Goodenough and Revel, 1970), tight junctions (Bulger, 1971), gap junctions (Revel and Karnovsky, 1967; McNutt and Weinstein, 1970) and desmosomes (Wood, 1959; Kelly, 1966).

A recent review (Weinstein and McNutt, 1972), attempts to classify the different types of cell junctions according to structure and function. This classification recognizes two major groups, junctions with close membrane to membrane contact and those with membranes separated by a 150 to 350 Å interspace. The first group includes two major sub-types, the tight or occludens junction, characterized by fusion of the outer leaflets of the apposed plasma membrane and by a loose network of intramembranous fibrils and the nexus or gap junction, characterized by a 20 Å space between the apposed membranes with hexagonally arrayed intra-

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membranous subunits. The second group contains three different types of junctional specialization, two of which, the intermediate junction and the desmosome (Webber and Blackbourn, 1971), are seen in the parietal layer of Bowman's capsule. These two junctions are characterized by a 150 to 350 Å space between apposed plasma membranes, by an intercellular space containing electron dense material and by cytoplasmic fibrillar condensations.

Different functions have been proposed for these junctional specializations (Loewenstein, 1972; Weinstein and McNutt, 1972). The tight junction forms a local permeability barrier restricting passage of material between adjacent cells, the gap junction plays a role in direct cell-to-cell communications, and the adherens junctions (intermediate junction and desmosome) provide a region of particularly strong attachment.

We have previously described the types of junctional specialization found in the parietal layer of Bowman's capsule in the rat and have also advanced evidence which suggests that this layer may be permeable to molecules at least as large as horseradish peroxidase (Webber and Blackbourn, 1971). The studies reported here are intended to compare the structural features of the junctions in this layer in man and rat to see if the rat is a useful model of the human structure. They are also designed to compare the development of the junctional region in the two species to ascertain whether significant changes in permeability would be expected on morphological grounds during the course of renal development.

Materials and Methods

Rat Kidney. These experiments were carried out on fetuses and newborn albino rats of the Wistar strain. In those experiments dealing with fetal tissue, the pregnant rats were anesthetized with a combination of sodium pentobarbital and sodium phenobarbital. The fetuses were removed and killed by decapitation. In the experiments dealing with newborn rats the animals were killed by decapitation.

The kidneys were removed, cut into appropriate sized blocks, fixed for three hours in 1 : 1 Karnovsky's fixative (Karnovsky, 1965), and osmium tetroxide in 0.1 M cacodylate buffer for one hour and treated in block with a saturated aqueous solution of uranyl acetate for one hour. The tissue was embedded in epon and sections cut using conventional techniques (Webber and Blackbourn, 1971). Thick and thin sections were then examined by light microscopy and electron microscopy using a Phillips EM200.

Human Kidney. The human tissue was obtained from hysterotomies during pregnancies having lasted more than 12 weeks and less than 20 weeks. As soon as the fetuses were removed, the kidneys were excised and cut into appropriate sized blocks, fixed for three hours in 1 : 1 Karnovsky's fixative and 0.2 M cacodylate buffer at room temperature and postfixed in two per cent osmium tetroxide in 0.1 M cacodylate buffer for one hour. They were then embedded and sectioned for examination by light and electron microscopy in the same manner as the rat tissue.

Results

Rat Kidney. In the parietal layer of developing Bowman's capsule, the cells presented a cuboidal appearance although the height of the cells often varied somewhat in the same glomerulus. The nuclei are large and oval in shape (Fig. 2).

The cytoplasm is abundant, the Golgi apparatus and the rough endoplasmic reticulum are well developed, the cytoplasm is rich in free ribosomes and the

mitochondria are numerous, large and often concentrated in one region of the cell where they assume a parallel orientation. There are usually some membrane-enclosed vesicles some of which may be pinocytotic. The cytoplasm close to the basement membrane often contains small bundles of fibrillar material similar to that described in the adult rat (Webber and Blackbourn, 1971). The distinction between apical and basal cytoplasm is less well defined in the immature renal corpuscle. The basal cytoplasm of the parietal epithelial cells contains more numerous organelles such as mitochondria and free ribosomes, which are sparse in the mature structure (Peace, 1968). The surface of the cells commonly shows irregular microvilli and quite often cilia. The nuclei have delicate chromatin with a few condensations close to the nuclear membrane (Fig. 1).

Most of the kidneys examined show few or no mitotic figures in the parietal layer, although they were usually quite abundant in the tubules (Fig. 2). In the sections in which there is a mitotic figure in the parietal epithelium, the junctions between this cell and the adjacent non-dividing cells are similar to the junctions between two non-dividing cells.

The intercellular junctions are most frequently seen in regions where the cytoplasm is quite thick. They usually have an almost straight course, although quite commonly it becomes somewhat tortuous close to the basement membrane. In this basal region junctional specializations are unusual and the membranes are separated by 300 Å or more (Fig. 1). Neither tight junctions nor gap junctions are positively identified in the parietal layer. Rarely a region which could be a tight or a gap junction was observed in the apical part of the cellular junction (Fig. 3).

The most frequent specialization encountered in the apical region was the intermediate junction. In these areas the gap between the cellular membranes is slightly less than 150 Å (Fig. 4) and was narrower than typical intermediate junctions. There is no fusion of the adjacent cell membranes that are parallel at the level of the junction. The intercellular space at that level is occupied by an amorphous material of low density. In the subjacent cytoplasm, a condensation of dense material is always present but this condensation varied in amount in different sections of the junctions. Two succeeding intermediate junctions separated by a stretch of membrane without junctional specialization were quite often seen (Fig. 4).

Desmosomes were also frequently seen. They presented the typical appearance of approximation of the two cell membranes, presence in the intercellular space of an amorphous material of low density with a band of higher density parallel to the cell membrane, condensation in the subjacent cytoplasm of dense fibrillar material within which there is a denser band underlying the cell membrane. The desmosomes were always seen in association with other junctional specialization of the intermediate junction type (Fig. 5).

Usually the junctional specialization does not reach the very apical region of the cellular junction, which consists of the two cell membranes slowly diverging towards the free surface of the cells. These regions are often overlayed by marginal folds of cytoplasm similar to that described in endothelial cell junctions (Fawcett, 1963). These cytoplasmic processes vary in length and width. The cytoplasm within these folds does not show any specialization. It was not uncommon to find a

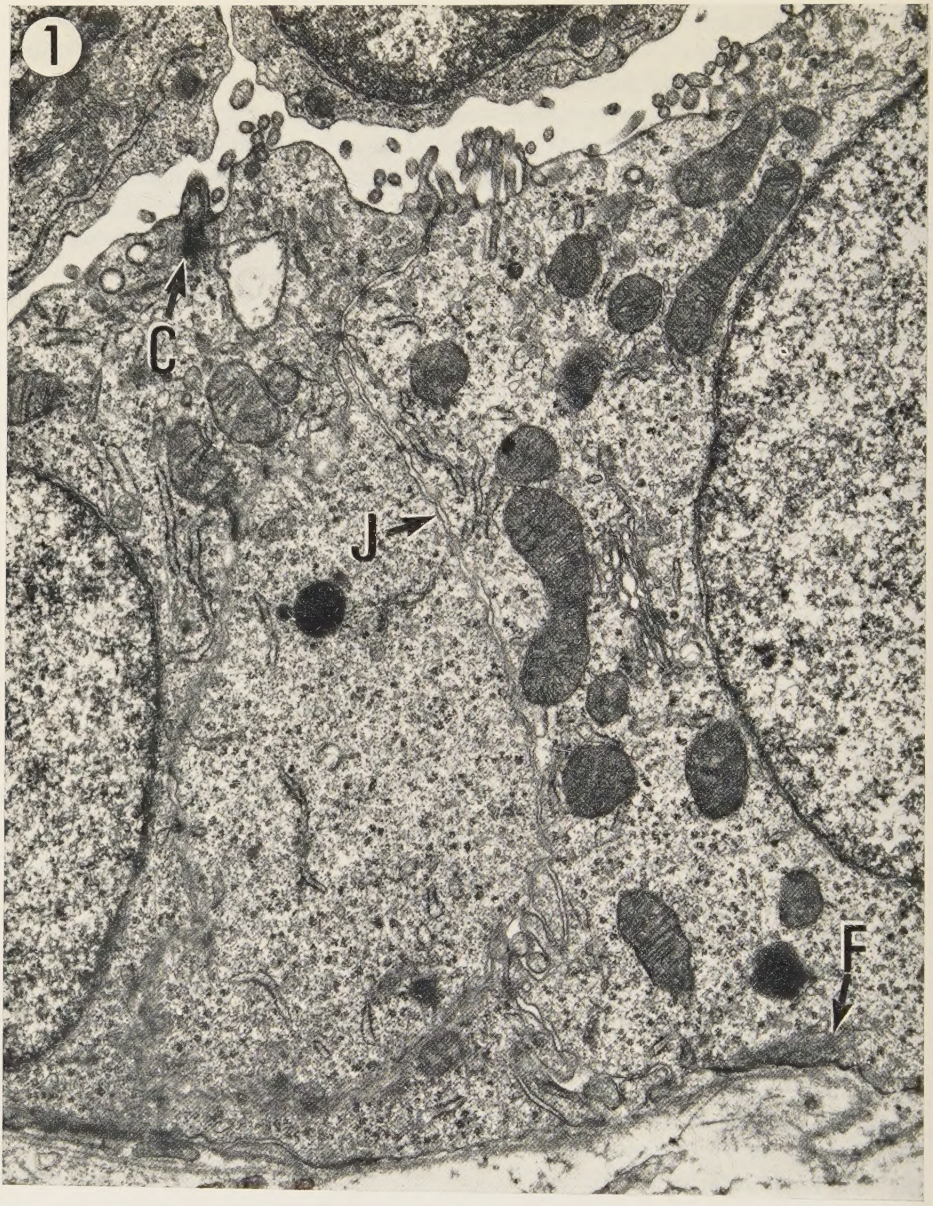
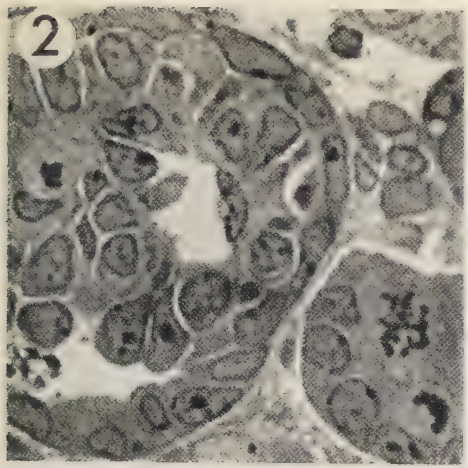


Fig. 1. Developing rat kidney showing the parietal layer of Bowman's capsule. Labelled features include cellular junction (*J*), base of cilium (*C*), and basal fibrillar material (*F*). Electron Micrograph (E.M.) ×18000

membrane bounded vesicle close to the cell junction. Such vesicles and marginal folds suggest a process of macropinocytosis in the parietal epithelium of Bowman's capsule (Figs. 1, 4) again resembling observations in endothelium.



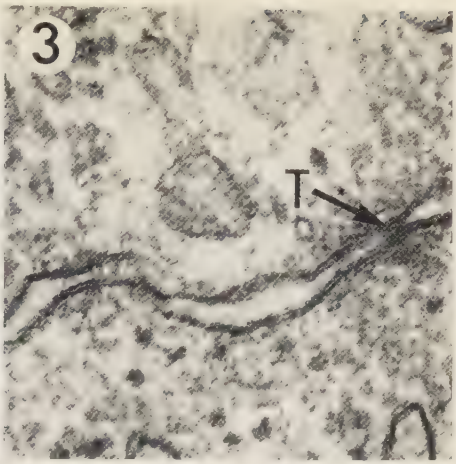
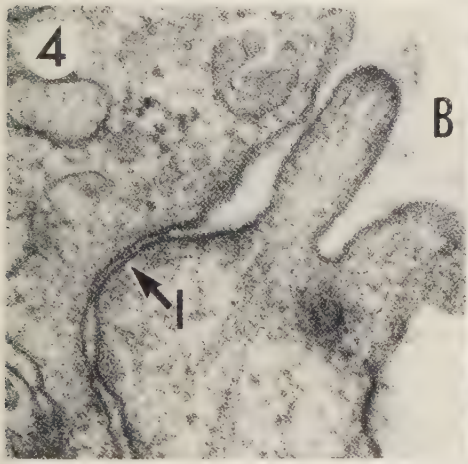


Fig. 2. Developing rat kidney showing a glomerulus with surrounding visceral and parietal layers of Bowman's capsule and adjacent tubules. Light micrograph ×3350

Fig. 3. Developing rat kidney showing a region of close apposition of the adjacent cell membranes (*T*). E.M. ×74000



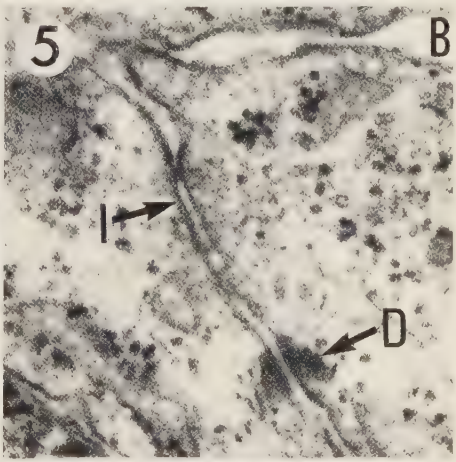


Fig. 4. Developing rat kidney showing Bowman's space (*B*) and regions of intermediate junction (*I*). E.M. ×67000

Fig. 5. Developing rat kidney showing Bowman's space (*B*), a region of intermediate junction (*I*), and a desmosome (*D*). E.M. ×67000

Human Kidney. The general appearance of the fetal human kidney is very similar to that of the developing rat kidney, although the human tissue has a more delicate structure. The stroma in the human tissue is much more extensive and in comparison to that in rat tissue was loose and pale staining.

The morphological differentiation of the renal corpuscles followed a similar course and the cells of the parietal epithelium are very much alike in the human

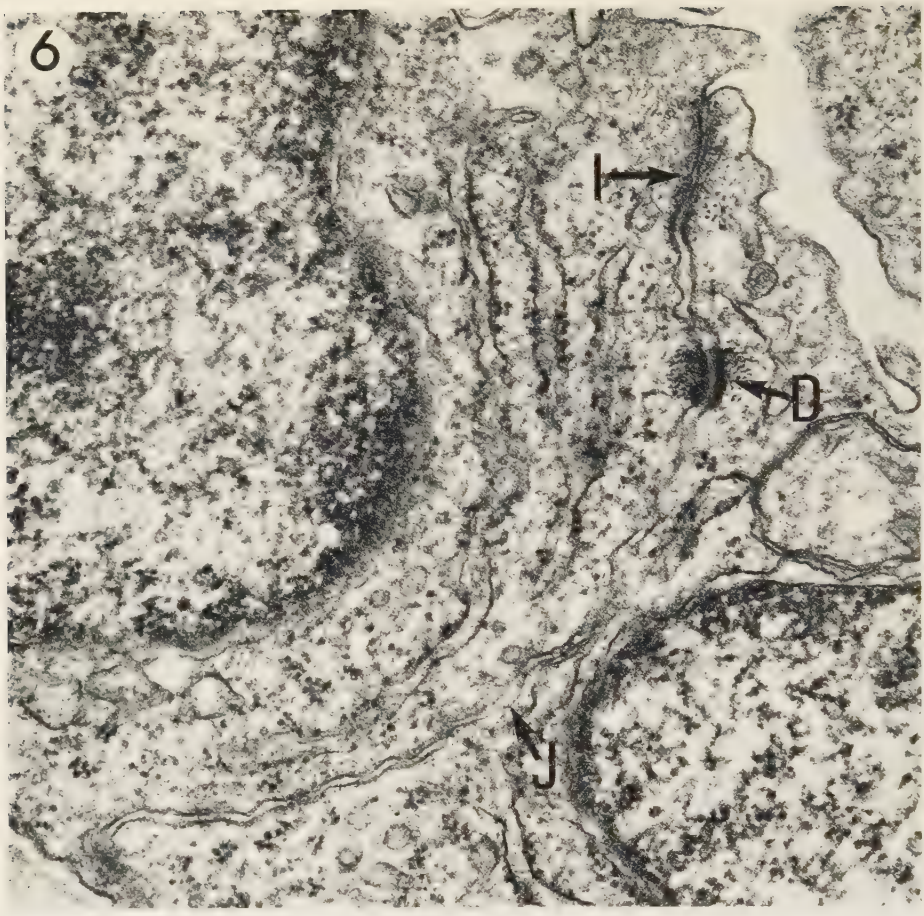


Fig. 6. Human fetal kidney showing a cellular junction (*J*) with a region of intermediate junction (*I*) and a desmosome (*D*). E.M. ×32000

and in the rat kidney, although in the human kidney the height of the cells in the same renal corpuscle tends to be more regular. Bundles of fibrillar material are sometimes present in the basal cytoplasm although it is less prominent than in rat tissue. By comparison with adult tissue there is less basal fibrillar material and more cellular organelles in the basal region.

The cellular junctions follow a straight course (Fig. 6). Quite often it is interrupted in the basal region by wide irregular empty spaces. The junctional specializations is prominent in the apical region. They are usually absent in the basal region, or, when present, consisted of a very narrow region of approximation of the cell membranes with no apparent cytoplasmic contribution. No tight or gap junctions were seen. The most frequent specializations were intermediate junctions but, as in the rat, with a narrower gap between the cellular membranes than has usually been described. These junctional specializations were always seen in the apical region of the cell (Figs. 6, 7) where they stretched over a longer distance

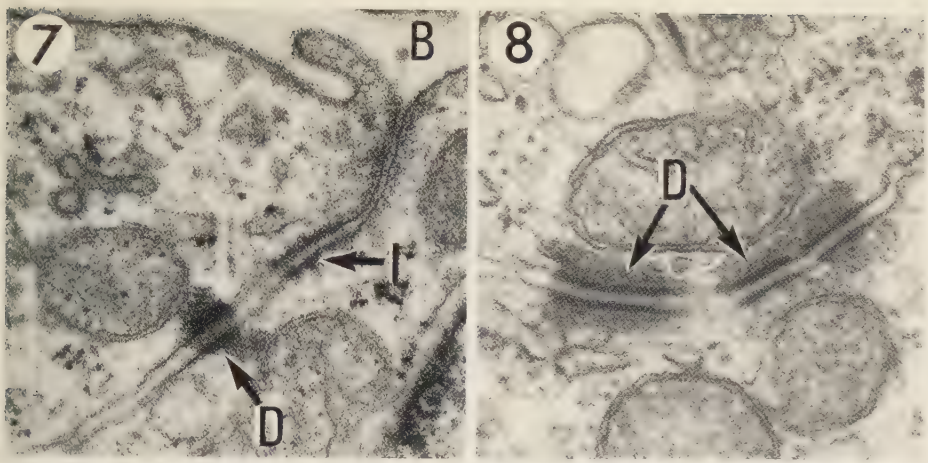


Fig. 7. Human fetal kidney showing Bowman's space (*B*), a region of intermediate junction (*I*), and a desmosome (*D*). E.M. ×45000

Fig. 8. Human fetal kidney showing two successive desmosomes (*D*) E.M. ×65000

than in the rat kidney. The usual pattern consists of two or more succeeding intermediate junctions, usually of different size. Desmosomes are regularly encountered and are always located deep to an intermediate junction (Fig. 7). Some cellular junctions present a common pattern: a succession of intermediate junctions and desmosomes separated by stretches of apposed membrane without junctional specialization (Fig. 8). Marginal folds overlying a junction are very common (Figs. 6, 7), and sometimes there was the appearance of a forming vesicle.

Discussion

In these experiments, we studied the junctional patterns in the parietal layer of Bowman's capsule from the developing renal corpuscles in rat and human fetal kidney. The maturation of the kidney in the rat has been described by Kittelson (1917), Arataki (1926), Zolnai (1965) and in the mouse by Rugh (1968). As far as the renal corpuscles are concerned, they first appear during fetal life about the 15th day of pregnancy (Rugh, 1968). New renal corpuscles are then formed up to the third week after birth, after which no more developing renal corpuscles can be found (Kittelson, 1917). The renal corpuscles in the rat continue to grow in diameter rapidly up to 150 days after birth and more slowly up to 500 days after birth (Aratcki, 1926).

The renal corpuscles are formed by vesicles which develop from small areas of metanephrogenic tissue clustered around the terminal branches of the collecting tubules (Aoki, 1966) and by vascular elements which invade the metanephrogenic tissue. The afferent arteriole grows first into the metanophrogenic tissue. Then the capillary loops differentiate and from them the efferent arteriole finally buds outwards. The walls of the vesicle grow and come to enclose the vascular part of the renal corpuscle (Arey, 1965). A portion of the walls of the vesicle will form Bowman's capsule with its two layers, visceral and parietal (Aoki, 1966).

Since new generations of glomeruli develop progressively in a centrifugal direction (Arey, 1965), the more differentiated glomeruli are found towards the medulla and the less mature close to the surface of the renal cortex. Glomeruli at various stages of development are observed in the same kidney in the rat fetus near birth and in the newborn rat (Webber and Blackbourn, 1970). As the age of the animal does not determine the degree of development of the glomeruli, we considered as immature glomeruli that have few capillaries, in which there was little endothelial fenestration, in which the podocytes had undeveloped podocytic processes (Fig. 2), and in which Bowman's space was small. The development of the glomeruli in the human kidney follows a basically similar pattern to that in the rat kidney (Aoki, 1966; Arey, 1965), but differs in timing in that at birth renal development is more advanced in the human.

In this study, the difference between the junctional patterns of the parietal epithelium of Bowman's capsule in developing rat and human fetal kidney was slight. The parietal epithelium in the human fetal kidney was characterized by its regularity, even size of cells and recurring patterns of junctional specialization. In both species, the cellular junctions followed a straight course in contrast to the tortuous path seen in adult tissue. The junctional specializations were located towards the luminal end of the cell junction and they were of similar type.

According to the frequency with which we encountered the different types of junctional specialization, we may say that in the parietal epithelium of Bowman's capsule from developing rat and human kidney tight junctions and gap junctions are probably not present or are very rare, while intermediate junctions and desmosomes are common. It is interesting to see that in this developing tissue the most commonly encountered specializations are those thought to provide strong attachment between two cells, and that the gap junctions commonly associated with cell-to-cell communication, and the tight junction forming a permeability barrier could not be shown with certainty.

These findings are similar to those that have been reported in the adult rat (Webber and Blackbourn, 1971). As in the mature renal corpuscle, the structure of the parietal epithelium of Bowman's capsule of the developing rat and human kidney probably allows a significant degree of permeability of this cell layer, since the minimum gap between the membranes of adjacent cells is greater than 100 Å. It is unlikely that the permeability characteristics of the layer change greatly during development since the only structural change is in the degree of tortuosity of the junctions. The type of junctional specialization appears to be characteristic of the tissue, to develope very early and to be similar in both species studied. It appears, therefore, that the rat kidney provides a useful model of the human kidney and that observations on permeability are likely to be transferrable between the two species.

References

Aoki, A.: Development of the human renal glomerulus. Differentiation of the filtering membrane. Anat. Rec. **155**, 339–351 (1966)

Arataki, M.: On the postnatal growth of the kidney with special reference to the number and size of the glomeruli (albino rat). Amer. J. Anat. **36**, 399–436 (1926)

Arey, L. B.: Developmental Anatomy. A textbook and laboratory manual of embryology, 7th ed., p. 303–304. Philadelphia: Saunders 1965

Bulger, R. E.: Ultrastructure of the junctional complexes from the descending thin limbs of the loop of Henle from rats. Anat. Rec. **171**, 471–475 (1971)

Farquhar, M. G., Palade, G. E.: Junctional complexes in various epithelia. J. Cell Biol. **17**, 375–412 (1963)

Fawcett, D. W.: Comparative observations on the fine structure of blood capillaries, chapt. 2, The peripheral blood vessels, Obison and Smith, eds., p. 17. Baltimore: Williams & Wilkins 1963

Goodenough, D. A., Revel, J. P.: A fine structural analysis of intercellular junctions in the mouse liver. J. Cell Biol. **45**, 272–290 (1970)

Karnovsky, M. J.: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. **27**, 137A–138A (1965)

Kelly, D.: Fine structure of desmosomes, hemidesmosomes, and an adepidermal globular layer in developing newt epidermis. J. Cell Biol. **28**, 51–72 (1966)

Kittelson, J. A.: The postnatal growth of the kidney of the albino rat, with observation on an adult human kidney. Anat. Rec. **13**, 385–408 (1917)

Loewenstein, W. R.: Cellular communication through membrane junctions. Arch. intern. Med. **129**, 299–305 (1972)

McNutt, N. S., Weinstein, R. S.: The ultrastructure of the nexus. A correlated thin section and freeze-cleave study. J. Cell Biol. **47**, 666–688 (1970)

Pease, D. C.: Myoid features of renal corpuscles and tubules. J. Ultrastruct. Res. **23**, 304–320 (1968)

Revel, J. P., Karnovsky, M. J.: Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. J. Cell Biol. **33**, C7–C12 (1967)

Rugh, R.: The Mouse: Its reproduction and development, p. 276. Minneapolis: Burgess Publ. Co. 1968

Webber, W. A., Blackbourn, J.: The permeability of the immature glomerulus to large molecules. Lab. Invest. **23**, 1–7 (1970)

Webber, A. W., Blackbourn, J.: The permeability of the parietal layer of Bowman's capsule. Lab. Invest. **25**, 367–373 (1971)

Weinstein, R. S., McNutt, N. S.: Current concepts. Cell junctions. New Engl. J. Med. **286**, 521–524 (1972)

Wood, R. L.: Intercellular attachment in the epithelium of *Hydra* as revealed by electron microscopy. J. biophys. biochem. Cytol. **6**, 343–352 (1959)

Zolnai, B., Palkovits, M.: New data concerning the growth of the glomeruli in the albino rat. Acta biol. Acad. Sci. hung. **15**, 393–408 (1965)

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